PCR Amplification using Taq Polymerase

Aim

To amplify gene of interest (BioBricks) for further analysis and/or experiments.

Procedure

Recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (95°C).

1. Thaw 10x CoralLoad PCR Buffer or 10x PCR Buffer, dNTP mix, primer solutions and 25 mM $MgCl_2$ on ice. Keep solutions on ice after complete thawing and mix thoroughly before use to avoid localized differences in salt concentration

2. Prepare a master reaction mix according to Table 1. Mix will contain all components needed except the template DNA. Calculate the appropriate volumes yourself - it is recommended to add the volumes for one extra reaction for each 8 reactions, e.g. for 16 reactions prepare a master mix sufficient for 18 reactions.

Table 1: Reaction Mix for total reaction volume of 100μl

Component	Volume/Reaction [μl]	Final concentration
Reaction Mix 10x (CoralLoad) PCR Buffer	10	1x
dNTP mix (10 mM of each nucleotide in mix present)	2	200 mM of each dNTP in mix
Taq DNA polymerase	0.5	2.5 units/reaction
Forward Primer	See end of protocol	0.2 μM
Reversed Primer	See end of protocol	0.2 μM
RNAse free water	Fill up to 100	

Table 2: PCR Thermocycler Program

Step	Temperature [°C]	Time [min]
Initial Denaturation	94	3
30 Cycles	94	1
	50	1
	72	1
Final Extension	72	10
Hold	4	N/A

3. Mix the reaction mix gently but thoroughly, by pipetting up and down a few times.

4. Place a 96 well plate into a ice bucket as a holder for the 0.2 ml thin walled PCR tubes. Transfer reaction mix to a PCR tube. Allowing the PCR reaction mix to be added into cold 0.2 ml thin walled PCR tubes will help prevent nuclease activity and nonspecific priming.

5. For each PCR tube add 1 μg of your DNA template, i.e. 1 μg DNA template/ reaction. For each reaction set up, make a negative and positive control with a PCR tube containing:
a) all PCR components except the DNA template, our **negative control** (No template control, NTC), fill the missing volume with water.

b) all PCR components with a DNA template you know will be amplified if reaction goes as planned with same primers as in your sample, our **positive control** (if standard template DNA is available)

6. Program the thermocycler according to the conditions in Table 2. Annealing temperature should Approximately be 5 °C below Tm of primers.For PCR products longer than 1 kb, use an extension time of 1 min per kb DNA. Put caps on the 0.2 ml thin walled PCR tubes and place them into the thermal cycler. Once the lid to the thermal cycler is firmly closed start the program.

7. When the program has finished, the 0.2 ml thin walled PCR tubes may be removed and stored at 4 °C.

8. PCR products can be detected by loading aliquots of each reaction into wells of an agarose gel then staining DNA that has migrated into the gel following electrophoresis.

Primers

Amount of Primers volume/reaction for primers calculate based on primer concentration. If VF2_R and VF2_F **working stock** are used, it is 2 μl per reaction for each of the primers

Sources

Qiagen quick-start PCR protocol.
Thermo fisher PCR protocol .
iGEM Bordeaux 2015 protocol.